Docket No.: 415072000101 (PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Jennie P. MATHER et al.

Application No.: 10/672,878

Confirmation No.: 9515

Filed: September 26, 2003

Art Unit: 1644

For: COMPOSITIONS AND METHODS FOR GENERATING MONOCLONAL ANTIBODIES REPRESENTATIVE OF A SPECIFIC CELL TYPE

Examiner: Y. Kim

DECLARATION UNDER 37 C.F.R. § 1.132

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Dear Sir,

I, Jennie P. Mather, declare as follows:

1. I have a Ph.D. in biology from the University of California at San Diego. I was an assistant professor at The Rockefeller University (1979-1984). I was a Senior Scientist (1984-1988) and a Staff Scientist (1988-1998) and head of the Cell Biology Group at Genentech, Inc. in South San Francisco, California. I am currently the founder, President and CSO of Raven biotechnologies, inc. in South San Francisco. I have had over 30 years of experience in cell culture. During this time, I have been an inventor on at least 28 issued patents and an author on over 150 publications, and have co-authored the book Introduction to Cell and Tissue Culture: Theory and Technique, Mather, J.P. and Roberts, P.E., Plenum Press, New York, NY 1998.

2. I am an inventor of the invention that is the subject of a patent application entitled Compositions And Methods For Generating Monoclonal Antibodies Representative Of A Specific Cell Type (hereinafter "the Application"), and am familiar with the contents of that Application.

3. The Application describes a method of producing monoclonal antibodies against cell surface antigens. Cell surface antigens (CSAs) are molecules anchored on the cell plasma membrane. CSAs constitute a large family of proteins, glycoproteins, polysaccharides and lipids, that serve not only as structural constituents of the plasma membrane, but more importantly, as regulatory elements governing a variety of biological functions. CSAs have been identified, cloned and found to play a pivotal role in the transduction of signals triggered by external stimuli such as growth factors and hormones that culminate in a wide range of cellular responses. Among them are cell division, differentiation, motility, and cell death. Defects in cell surface antigens, such as receptors and adhesion proteins in particular, are now known to account for a vast number of diseases, including numerous forms of cancer, vascular diseases and neuronal diseases.

4. Before our invention, the pools of monoclonal antibodies generated by practice of the commonly known methods were not necessarily or predictably directed against the CSAs of the cells of interest, or if they were, it was not predictable that they would recognize the antigens in their native conformation. As tools for research on CSAs, or for therapeutic or diagnostic purposes, the populations of antibodies generated prior to this invention were not likely to be predictably directed against the antigens representative of a particular cell surface, or representative of those appearing on a particular tissue (or sub-tissue) type.

5. It was our desire to discover a method of generating monoclonal antibodies reactive with the antigens that are present on the surface of cells, or that are representative of a particular cell type, that was substantially better than what was known and practiced in the art at the time of invention.

6. For a number of years prior to our invention, researchers investigated CSAs by making membrane extracts of some cells of interest, or taking intact cells of a particular cell type that have been grown in a serum-supplemented medium, and then using these extracts or cell preparations in traditional methods as immunogens to generate pools of monoclonal antibodies. These methods often did not achieve the desired result. Problems existed with the traditional methods used to prepare the immunogens for monoclonal antibody production. Extraction of the surface antigens from a cell involved detergents or other organic solvents that were known to disassemble the plasma membrane bilayer and thus dissociate the surface antigens from their native environment and alter their native conformation. If the researcher used intact cells grown in serum-supplemented media, then immunization using those cells yielded a substantial population of resulting monoclonal antibodies that were reactive against serum biomolecules and other contaminants, rather than against a CSA.

7. At the time of this invention, there remained a considerable need for compositions and methods for generating a population of monoclonal antibodies that specifically binds to antigens representative of a particular cell type. The invention described in the application satisfies these needs, and the claimed methods yield populations of monoclonal antibodies that significantly represent the surface antigens of a desired cell type.

8. This invention teaches techniques of antibody production that minimizes the generation of non-representative antibodies cross-reacting with proteins not present in a particular cell type, or on the surface of a cell of interest. These techniques also maximize the preservation of intact antigens, especially surface antigens, for production of a plurality of monoclonal antibodies that bind to the native antigens of a particular cell type. These methods, and the resulting antibodies, greatly facilitate the identification and exploration of novel antigens.

9. There are several key features of the present invention, involved in the ways we discovered to introduce into a host mammal a plurality of viable and intact cells,

using those cells to immunize the host mammal to produce a population of monoclonal antibodies that bind to antigens representative of a specific cell type that are heterologous to that host mammal.

10. One key feature of the present invention lies in the use of viable and intact cells as the immunogen, instead of denaturing the cell membrane to extract surface antigens as discussed above, and as was commonly practiced at the time.

11. In addition to our use of viable and intact cells as the immunogen, another key feature of the present invention likes in the use of cell surfaces that were free of serum. The Application describes several ways of achieving this serum-free status.

12. Another key feature of this invention involves the way we prepared the immunogen. The Application teaches the method of preparing the immunogen in order to generate monoclonal antibodies without the use of adjuvant. Our methods can be distinguished from the methods then-known and practiced in the art.

13. At the time of our invention, doctrine and standard practice suggest boosting a host animal's immune response to an immunogen by immunizing an animal with the immunogen plus Freund's adjuvant. The typical protocol at the time of this invention can be seen in Exhibit 1, attached hereto. Exhibit 1 shows a protocol for immunizing mice in order to generate monoclonal antibodies, taken from the standard treatise Current Protocols in Immunology. This protocol is representative of what was commonly practiced and known in the art at the time of our invention. Expressly listed in the compounds required for immunization is the adjuvant, "Complete Freund's adjuvant". It was not typical at the time to avoid adjuvant entirely as taught in the Application.

14. I believe that the excellent and unexpected results of the practice of this invention, as described in the Application, arise from the combination of features of the claimed invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements

were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Sept 23 2005
(Date)

Jennie P. Mather, Ph.D.

UNIT 2.5

Production of Monoclonal Antibodies

Highly specific antibodies can be obtained by fusing immune B cells from the spleen with tumor cells to produce hybridomas, each of which will then secrete a single antibody. The desired antibody-producing hybridoma can be identified by a screening process. If this hybridoma is subjected to a cloning process in which clones are selected, such that all progeny are derived from a single cloned parental cell, a monoclonal antibody is obtained. Monoclonal antibodies have high specificity and can be produced in large quantities. Thus, these biological reagents have been used extensively as probes in a wide range of systems including the characterization of novel cell-surface and soluble proteins and carbohydrates, as enzyme catalysts, and for targeting in immunotherapy (see commentary).

This unit describes the production of monoclonal antibodies beginning with basic protocols for immunization and cell fusion and selection.Support protocols are provided for screening primary hybridoma supernatants for antibodies of desired specificity, establishment of stable hybridoma lines, cloning of these B cell lines by limiting dilution to obtain monoclonal lines, and preparation of cloning/expansion medium (thymocyte-conditioned medium). Figure 2.5.1 summarizes these stages and notes the protocols in this and subsequent units in which they are detailed. A major commitment of time and labor is necessary but, if successful, the monoclonal antibody may be an extremely valuable reagent that will be available in large quantities.

Submission of monoclonal antibodies to the American Type Culture Collection (ATCC) for distribution to the scientific community is encouraged. Moreover, the ATCC serves as a repository for cell lines should the line be lost in the investigator's laboratory due to unforeseen circumstances.

NOTE: Sterile technique must be used in all procedures that utilize tissue culture cells.

BASIC PROTOCOL

IMMUNIZATION TO PRODUCE MONOCLONAL ANTIBODIES

A wide variety of antigen preparations have been used successfully to produce monoclonal antibodies (see critical parameters discussing antigen preparation). The following protocol provides an immunization schedule for the production of most antibodies, although several different schedules can be used. In this protocol, emulsified antigen is injected intraperitoneally into the species of choice. A booster injection is administered 10 to 14 days after the primary immunization. Three days after the booster injection, the animals' spleens are ready for cell fusion (second basic protocol).

Materials

Antigen
Complete Freunds adjuvant (CFA; Sigma)
Animal: pathogen-free mouse, hamster, or rat (Armenian hamsters from Cytogen Research are recommended; see critical parameters for discussion of animal choice and *UNIT 1.1*)
Incomplete Freunds adjuvant (IFA; Sigma), optional
1- to 2-ml glass syringes with Luer-Lok tips, sterile
3-way stopcock
20- and 22-G needles, sterile
Additional reagents and equipment for handling and restraint of animals (*UNIT 1.3*) and intraperitoneal injection (*UNIT 1.6*)

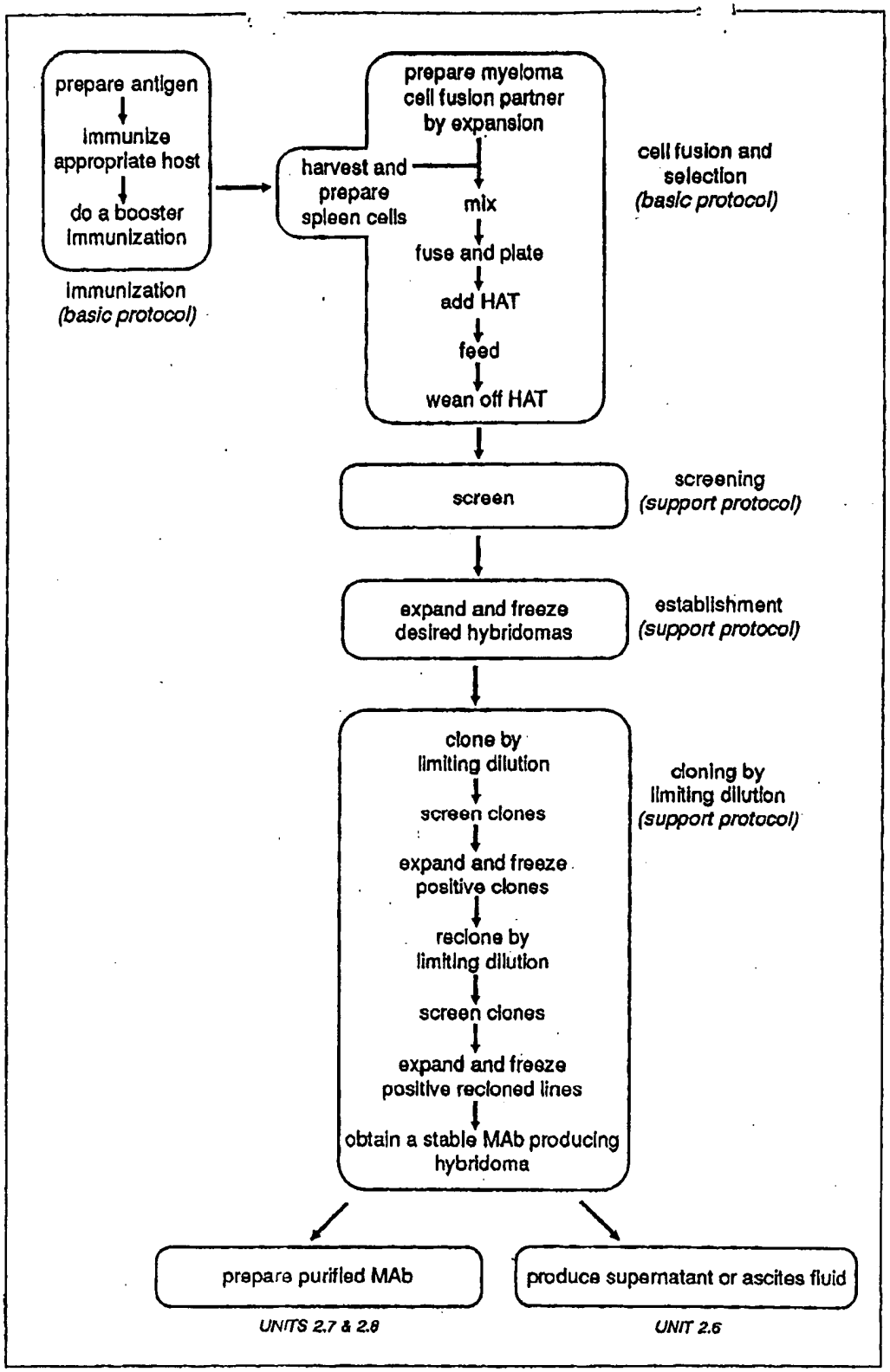

Figure 2.5.1 Stages of monoclonal antibody production, with references to the basic and support protocols in this unit (as well as subsequent units) that describe the steps.

CAUTION: CFA is an extremely potent inflammatory agent, particularly if introduced intradermally or into the eyes. Profound sloughing of skin or loss of sight may occur. Self-injection can cause a positive TB skin test and lead to a granulomatous reaction. Use gloves and protective eyewear when handling CFA.

1. Prepare antigen using 2×10^6 to 5×10^7 cells or 1 to 50 μg protein or peptide per animal to be immunized in normal saline.

   The antigen may be in several different forms depending on the desired property of the MAb and the method of screening (see critical parameters for discussion of antigen preparation and screening assays). If cells are the immunogen, wash three times in serum-free medium before immunization. Plan the immunization of several animals (enough for several fusions) so that primed and boosted animals will be ready 3 days before fusion (see second basic protocol).

   To minimize the risk of introducing a pathogen into the rodent colony, screen cells for pathogens by antibody-production assay (UNIT 1.1).

2. Draw up antigen into a sterile 1- to 2-ml glass syringe with a Luer-Lok tip. Connect syringe to a 3-way stopcock.

3. Completely resuspend CFA to disperse the *Mycobacterium tuberculosis* bacilli which settle to the bottom of the container with time. Draw up a volume of CFA equal to the antigen volume in a syringe and connect to the antigen-containing syringe.

4. Emulsify antigen and CFA by discharging antigen into CFA, then discharging back and forth until a thickened mixture results. Test whether the emulsion is stable—a stable emulsion will not disperse when a drop of it is placed in water.

   See UNIT 2.4 for further discussion of immunization. Figure 2.4.1 illustrates the double-syringe device.

5. Transfer all of the CFA/antigen emulsion to one syringe and remove the other syringe and stopcock. Attach a sterile 20-G needle to the syringe containing the emulsion.

6. Inject emulsion intraperitoneally into the animal using <0.2 ml/mouse, 0.5 to 1 ml/rat, or 0.2 to 0.4 ml/hamster.

   Be careful not to force the syringe plunger since excessive pressure may dislodge the needle and spray the emulsion. Introduce the needle through the skin and tunnel the needle between the skin and peritoneal wall before entering the peritoneal cavity at a site distant from the dermal puncture site. Twirl needle before withdrawal to minimize leakage.

   Rats are generally anesthetized (UNIT 1.4) whereas mice and hamsters can be manipulated with one hand and do not require anesthetic.

7. Boost animal after 10 to 14 days with approximately the same dose of antigen as in step 5. If cell fusion is planned for 3 days after boosting, immunize with antigen alone in aqueous solution, or intact cells in suspension. If a fusion is not immediately planned, boost the animal with antigen emulsified in IFA (which does not contain *Mycobacterium tuberculosis* bacilli).

   Do not use CFA for the booster immunizations as this will cause intense inflammation and increased anti-TB antibody response.

   If desired, antibody titers can be assayed by ELISA (UNIT 2.1) or immunoprecipitation (UNIT 8.3), 7 to 10 days after the primary and booster immunizations.